REMARKS

Claims 1-54 are pending in this application, with claims 1-24, 42 and 43 withdrawn from consideration. A number of claims have been amended as shown above. Claims 25 and 26 have been amended to clarify the scope of the claims. Support for the amendments can be found, for example, at pages 15-24 of the specification as filed. Claims 27-29, 34-36, 47-48, 51 and 53-53 also have been amended to clarify the scope of the claims. Support for the amendments for "full-length cDNA transcript" can be found, for example, in claim 30 and at page 22, lines 11 and 15-16 of the specification. Support for the amendments to claims 39 and 40 can be found at page 22, lines 8-16 of the specification as filed. Support for the amendments to claim 41 can be found at page 16, lines 22-25, and page 22, lines 23-24.

Applicants note with appreciation that a number of rejections from the previous Office Action have been withdrawn in view of the amendments and arguments presented by Applicants.

A number of claims were objected to because of informalities. The objection to claims 27-28 because the word "a' is lacking has been obviated in light of the appropriate amendment to the claims above.

Claims 44-47 and 53 were objected to on the basis that claim 44 recites "nucleotide acid molecule" rather than "nucleic acid molecule." This objection has been rendered moot by the amendment above to claim 44.

Claim 35 was objected to on the basis that there was improper subject-verb agreement. Applicants have corrected this error.

Claim 37 was objected to on the basis that the word "a" was missing. This typographical error has been corrected.

Claims 25-41 and 44-54 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. More specifically, claim 25 was said to be indefinite because it was not clear whether the claim was drawn to a method for "preparing at least one oligonucleotide including at least one ditag," or to a method of simply creating "at least one ditag," as set forth in the final linking step of the claim. The claim also was

said to be indefinite in the recitation of "the nucleic acid molecule or fragment thereof," in the "isolating" step, on the basis that it was not clear how this recitation limited the claim. Applicants have amended claim 25 to clarify the steps of the method defined in, and to use consistent language throughout, the claim. Applicants respectfully submit that the examiner's concerns have been obviated by the amendments to the claim.

Claims 26-38 and 44-45 were said to be indefinite in that it was unclear whether the claims require preparation of "at least one oligonucleotide comprising at least one ditag (preamble of claim 26)," or of "at least one oligonucleotide including at least one ditag flanked by two adapters (final method step)." The examiner asserted that it was not clear what type of oligonucleotide(s) must be formed to meet the requirements of the claim. The examiner also asserted that the recitation "the oligonucleotide comprising" in claim 29 was unclear, as the claim depends from claim 26, which refers to "at least one oligonucleotide" but not to a single, specific oligonucleotide.

Claim 26 has been amended to clarify the steps of the claim. In particular, the wording of the claim clarifies the method of producing a ditag from a full-length cDNA transcript; i.e., that the 5' tag is produced with the adapter at the 5' end, the 3' tag is produced with the adapter at the 3' end, and the two tags are ligated to form a ditag flanked by two adapters. Applicants submit that this language is clear and consistent throughout. With regard to claim 29, this claim and the claims dependent from it now refer to "at least one ditag" rather than to an "oligonucleotide." Applicants submit that this amendment obviates the examiner's objection.

Claim 32 was said to be indefinite in the phrase "whereby matching 5' and 3' termini sequences are identified," as it was not clear what was encompassed by this limitation. Claim 32 has been amended above to recite "mapping the 5' and 3' tags of the ditag nucleotide sequence to a database...." As a result of the preparation method, the two tags in each ditag are from the same full-length cDNA transcript and the orientation of the tags as mapped to the genome sequences are the same as in the ditag and the full-length cDNA transcript. Applicants respectfully submit that the method of mapping the ditags recited in the claims is clear to one of skill in the art.

Claims 33-36 were said to be indefinite in the recitation "splicing the 5' terminus and the 3' terminus of the molecule to produce at least one ditag, wherein splicing includes adding at least one restriction enzyme capable of recognizing the recognition sites." Claims 34-36 were said to be indefinite in the recitation of "the two recognition sites," because there was not proper antecedent basis for the phrase, and claim 35 was said to be indefinite in the recitation of "the type II restriction enzyme is ...," because there was no proper antecedent basis to a single particular enzyme.

Claim 33 has been amended above to clarify the steps of the method of independent claim 26. As amended, it is clear that step (ii) of claim 26 includes adding at least one restriction enzyme capable of recognizing the at least one restriction site to the full-length cDNA transcript from step (i) of claim 26. Each of claims 34-36 also has been amended to clarify the claims, thereby obviating the basis of each rejection.

Claim 37 was said to be indefinite on the basis that it was not clear how the claim further limited claim 26, from which it depends. It also was said to be indefinite in reciting "producing one full-length cDNA molecule comprising two adapters flanking the 5' terminus and 3' terminus" because it was unclear whether one or two adapters were required at each end of the molecule. Claim 37 has been amended to clarify the steps of the method of independent claim 26 and makes clear that step (ii) of that claims comprises cleaving the full-length cDNA transcript from step (i) with MmeI as the restriction site is an MmeI recognition site.

Claim 39 was said to be indefinite in the step "defining the structural region of the corresponding gene on the genome map." Applicants respectfully submit that the phrase is not indefinite. From page 22 of the specification, it is taught that the genomic DNA sequence in between the two tags of the ditag is the full structural content of the prospective gene, including the exons and introns. As discussed in more detail below, the method of the invention allows the delineation of the gene from the full-length cDNA transcript. One of skill in the art would consider that the mapping and full delineation of the gene also would allow the study of upstream and downstream regulatory regions such as promoters and enhancers, which also are important structural regions of the

gene. Accordingly, the reference to the structural region of the corresponding gene on the genome map is clear to one of skill in the art.

Claims 40 and 41 were said to be indefinite in the phrase "the obtained at least one ditag," because the claim does not refer to obtaining a ditag. The examiner suggested that the word "obtained" be stricken from the claim, and Applicants have made this amendment. These two claims also were said to be indefinite in the recitation of "and/or." Applicants have amended claim 40 to recite "and" rather than "and/or." Claim 41 further was said to be indefinite in reciting "recovering the full-length nucleic acid molecule corresponding to the newly discovered gene." The claim has been amended to recite "recovering the full-length cDNA corresponding to the ditag by PCR or directly from target RNA samples by RT-PCR." In view of this amendment, Applicants respectfully submit that the claim defines a method of recovering full-length cDNA corresponding to the ditag (referred to in claim 40) by PCR or RT-PCR.

Finally, claims 44-47 and 53 were said to be indefinite in reciting "the vector comprises an isolated oligonucleotide" in claim 44 because it is not clear how the oligonucleotide could be considered to be isolated. The examiner suggested that this term be deleted from the claim, and the Applicants have done so by amendment above.

Claims 25-31, 33-35, 37-38, 44-49 and 53 have been rejected under 35 U.S.C. §102(b) as anticipated by U.S.P. 6,136,537, issued to Macevicz (hereinafter referred to as Macevicz I). The examiner asserted that the specification of the present application does not provide a limiting definition of "ditag." He characterized the Macevicz I patent as disclosing a modification of the serial analysis of gene expression (SAGE) method in which pairs of sequence tags are constructed using a portion of each end of a target polynucleotide such as a cDNA. The patent was said to disclose a method in which a nucleic acid molecule of interest is inserted into a vector and subsequently cleaved, linearized and recircularized to form a tag pair or "ditag" including the 5' and 3' termini of the nucleic acid molecule and to disclose producing the nucleic acid molecules employed in their methods. This rejection is traversed.

Applicants respectfully submit that the claims as now amended clearly distinguish over the teachings of Macevicz I. The reference does not disclose a method of preparing ditags from a full-length cDNA transcript. Indeed, the reference notes at column 6, lines 22-23, that when the method disclosed is applied to cDNAs without cleavage by a restriction endonuclease it will result in one of the tags of each pair consisting of a segment of polyA tail which lacks information content.

The invention presently claimed relates to a method of producing ditags from the same full-length cDNA transcript, with the ditags providing sequence information from both ends of the full-length cDNa transcript. As set forth in the claims and as illustrated in figures 1 and 2 of the present application, the ditags comprise the 5' end and the 3' end of the same full-length cDNA transcript (see also page 21, lines 10-17 of the specification as filed). In addition, page 22, lines 11-12, of the specification as filed teaches that the paired 5' and 3' signature sequences (tags) of a transcript in a ditag sequence delineate the starting and ending points of transcripts, such that when the two tags are mapped head-to-head in a specific region on a chromosome of an assembled genome sequence, the genomic sequence in between these two tags is the full structural content of the prospective gene, including exons and introns.

The method of the present invention as claimed produces ditags which provide sequence information from both ends of the full-length cDNA transcript for delineating the entire coding region of a gene, including exons and introns. Such a method is not taught by Macevicz I and the reference, therefore, does not anticipate the pending claims.

Claims 25 and 39 have been rejected under 35 U.S.C. §102(b) as anticipated by U.S. Patent 6,054,276, issued to Macevicz (hereinafter referred to as Macevicz II). The examiner asserted that the reference discloses a method of genomic mapping and gene expression monitoring in which pairs of sequences obtained from each end of a restriction fragment are employed. The steps taught in Macevicz II of providing nucleic acids, excising the 5' and 3' end thereof and ligating them together were said to result in the formation of a molecule that constitutes at least one “ditag.” The reference also was

said to disclose the use of concatemers of multiple ditags obtained from nucleic acid populations including cDNAs and the use of such concatemers in mapping, which results in "defining the structural region of the corresponding gene on the genome map." This rejection is traversed.

As amended, claim 25 is directed to a method of preparing a ditag from a full-length cDNA transcript, wherein a 5' tag is cleaved from the 5' terminus of the transcript, a 3' tag is cleaved from the 3' terminus of the transcript and the 3' end of the 5' tag is ligated to the 5' end of the 3' tag to create the ditag. The reference does not disclose the preparation of ditags from the same full-length cDNA transcript and so it also does not anticipate claim 25.

Neither does the reference anticipate claim 39. The disclosure of the Macevicz II reference relates primarily to a method of constructing a physical map of a nucleotide. The mapping method is very different from that of the present invention in that it aligns the sequences obtained from the tags to each other to obtain a physical map (see figure 1 and column 3, line 65, to column 4, line 40). The physical map of the nucleotide provides scattered bits of sequences as shown in Figure 4 of the reference. The focus of the Macevicz II reference thus is a method of constructing a physical map of a nucleotide, rather than gene expression analysis. Gene expression analysis is mentioned briefly in the reference, but Macevicz II as applied to cDNA utilizes frequency to analyze the data (column 2, lines 40-46 and claims 5-6), and tag sequences are not used for genome mapping. The analysis method using frequency is expanded in the Macevicz I reference, which is a CIP of Macevicz II, (see column 4, line 52 - column 6), but it does not address genome mapping, as acknowledged by the examiner on page 14, second paragraph, of the outstanding Office Action. Neither reference, therefore, discloses genome mapping as required in claim 39. As mapping to the genome is quite different from the physical mapping of the reference, the reference does not anticipate claim 39.

Claim 32 has been rejected under 35 U.S.C. §103(a) as unpatentable over Macevicz I in view of Saha et al., *Nature Biotechnology* 19:50 (2002). The examiner

relied upon Macevicz I as in the rejection above. He acknowledged that the reference does not disclose comparing ditag sequences to a database comprising genomic sequences and identification of "matching" 5' and 3' termini. The reference by Saha et al. was cited as disclosing querying the human genome sequence database to determine the genes corresponding to tags, and the examiner concluded that as the tags of Macevicz I comprise the 5' and 3' termini of nucleic acid molecules being analyzed, the performance of the method of Saha et al. using the tags of Macevicz I would result in matching both 5' and 3' termini, as required by claim 32. This rejection is traversed.

The shortcomings of the Macevicz I reference have been discussed above, and that discussion is equally applicable to the present rejection. The reference does not teach a method for preparing ditags from a full-length cDNA transcript which delineates the entire coding region of a gene. The paper by Saha et al. does not compensate for the deficiencies of the primary reference. Saha et al. disclose the mapping of independent tags, and the method of the reference requires additional analysis to obtain full-length gene sequences (see page 510, the beginning of the second column). Taken in combination, the two references do not suggest the method of claim 32, which provides for obtaining the ditag from the full-length cDNa transcript and then mapping the 5' and 3' tags of the ditag nucleotide sequence to a database comprising genomic sequences.

Claim 36 has been rejected under 35 U.S.C. §103(a) as unpatentable over Macevicz I in view of Belfort et al., *Nucleic Acids Research* 25(17):3379 (1997). The examiner relied upon Macevicz I as in the previous rejections. He acknowledged that the reference does not disclose the use of asymmetric restriction sites that "are homing endonuclease asymmetric recognition site sequences" recognized by any of the enzymes set forth in claim 36. He asserted, however, that Belfort et al. teach that homing endonucleases are rare-cutting enzymes and that such enzymes include I-CeuI, PI-SceI, PI-PspI and I-SceI and that it thus would have been obvious to have modified

the method of Macevicz I so as to employ adaptors including restriction sites for any of the endonucleases taught by Belfort et al. This rejection is traversed.

The shortcomings of the Macevicz I reference have been discussed above, and that discussion is equally applicable to the present rejection. The cited secondary reference does not compensate for the deficiencies of th primary reference, and one of skill in the art reading the two references in combination would not arrive at the claimed invention.

Claims 40 and 41 have been rejected under 35 U.S.C. §103(a) as unpatentable over Macevicz II in view of Saha et al. The examiner relied upon Macevicz I as in the rejection under 35 U.S.C. §102, above. He acknowledged that the reference does not teach "detecting no match on one or more gene databases" as in claim 40 or the further step in claim 41 of "recovering the full-length nucleic acid molecule" corresponding to the discovered gene. He asserted that the secondary reference discloses querying the human genome sequence database to determine the genes corresponding to tags and the further analysis of unmatched tags that represent potential undiscovered genes and that Saha et al. teach that unmatched tags have "no match on one or more gene databases" as in current claim 41 and that PCR constitutes recovering a full-length nucleic acid molecule corresponding to a newly discovered gene as require in claim 41. He concluded that it would have been obvious to modify the teachings of Macevicz II so as to perform further steps as taught by Saha et al. This rejection is traversed.

The shortcomings of the Macevicz II reference have been discussed above, and that discussion is equally applicable to the current rejection. Macevicz II does not disclose a method of preparing ditags from a cDNA transcript corresponding to a full-length gene. The secondary Saha et al. reference does not compensate for the deficiencies of the primary reference. Saha et al. discloses a LONG-SAGE method which prepares single tags from each transcript, generally from the internal region of the transcript because the cDNA is first cut with a restriction enzyme, and the mapping of such tags. The end of the first column on page 510 of this reference discloses that genes can be missed if they do not contain a restriction site used initially to cut the

cDNA molecule and suggests the use of different restriction enzymes for the initial restriction. The reference thus not only does not compensate for the deficiencies of the primary reference, it teaches away from the presently claimed invention. The references considered in combination do not render obvious claims 40 and 41.

Claim 25 has been provisionally rejected on the ground of non-statutory obviousness-type double-patenting as being unpatentable over claims 1-19 and 24-25 of copending application No. 11/045,468. Applicants previously have requested that this rejection be held in abeyance until claims of the present application have been found to be in condition for allowance. If at that time the rejection still stands, Applicants will file a terminal disclaimer if appropriate.

Applicants respectfully submit that the claims are in condition for allowance.

Respectfully submitted,

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